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Do free-ranging common brushtail possums (*Trichosurus vulpecula*) play a role in the transmission of *Toxoplasma gondii* within a zoo environment?

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Abstract

To investigate the possible role of common brushtail possums (*Trichosurus vulpecula*) in the transmission of *Toxoplasma gondii* within a zoo environment, a serological survey of a free-ranging population resident within Taronga Zoo, Sydney, Australia was undertaken using the modified agglutination test (MAT). For comparison, the seroprevalence of *T. gondii* antibodies was also assessed in a possum population inhabiting a felid-free, non-urban woodland habitat. Six of 126 possums (4.8%) from the zoo population had antibodies to *T. gondii* with a MAT titre of 25 or higher, while in contrast, all of the 17 possums from woodland were seronegative. These observations suggest that possums were at a higher risk of exposure to the parasite as a consequence of co-existing with domestic, stray and captive felids associated with urbanisation. Screening of captive felids at the zoo indicated 16 of 23 individuals (67%) and all 6 species were seropositive for *T. gondii*, implicating them as a possible source of the parasite within the zoo setting. In addition captive, non-felid carnivores including the chimpanzee (*Pan troglodytes*), saltwater crocodile (*Crocodylus porosus*), dingo (*Canis lupis*) and leopard seal (*Hydrurga leptonyx*) were tested for the presence of *T. gondii* antibodies as these species predate and are a leading cause of death amongst zoo possums. In total, 5 of 23 individuals (22%) were seropositive, representing 2 of the 4 carnivorous species; the dingo and chimpanzee. These data suggest that carnivory was not a highly efficient pathway for the transmission of *T. gondii* and the free-ranging possum population posed minimal threat to the health of zoo animals.

Keywords: *Toxoplasma gondii*; Urban wildlife; Possum; Disease transmission; *Trichosurus vulpecula*

1. Introduction

Toxoplasma gondii, the causative agent of toxoplasmosis, is an intracellular parasite of medical and veterinary importance (Dubey and Beattie, 1988). The parasite is found worldwide reflecting the distribution of felids, the definitive host of *T. gondii*, within which the parasite undergoes sexual reproduction to produce the infectious 'oocyst' life stage shed in faeces (Dubey and Lappin, 1998; Tenter et al., 2000). *T. gondii* has also adapted to utilise warm-blooded animals, including birds and mammals, as an intermediate host. In these animals, tissue cysts are formed and remain viable over the lifespan of the animal (Dubey, 2004). Infection occurs via either consumption of oocysts, transplacental

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transmission or ingestion of tissues from an infected animal (Kreier and Baker, 1987). Toxoplasmosis can be life-threatening to young or immuno-compromised individuals and for animal groups that have not developed resistance to felid parasites, such as marsupials and New World monkeys, infection is often severe or fatal (Dubey and Beattie, 1988).

Contact with *T. gondii* has been recorded in an increasing range of wildlife that co-exist with humans in urban areas of Northern America (Smith and Frenkel, 1995; Dubey et al., 2004; Riley et al., 2004; Hancock et al., 2005) and Australia (Eymann et al., 2006). This data suggests that urbanisation may heighten interaction between wild animals and domestic or stray cats, providing opportunities for *T. gondii* to expand its host range. The habituation of wildlife such as the Virginia opossum, *Didelphis virginiana* (Harmon et al., 2005) and the raccoon, *Procyon lotor* (Junge et al., 2007) to anthropogenic food and shelter afforded within zoos, places them at risk of primary exposure to infections present in zoo animals including *T. gondii*. Often thriving in zoo environments, free-ranging wildlife may act as an important intermediate host of *T. gondii*, facilitating completion of the parasite's life-cycle through interaction and predation by captive felids (Riemann et al., 1974; Ippen et al., 1981). Despite free-ranging animals being implicated in the dissemination of *T. gondii* leading to fatalities of captive animals (Junge et al., 1992; Dubey et al., 2001; Spencer et al., 2004) the seroprevalence of wildlife that occupy zoo grounds has never been investigated. Screening for *T. gondii* may be an important pre-emptive measure to assess the risk of infection to captive collections, particularly as zoo animals increasingly represent valuable genetic stock for populations diminishing in the wild.

The common brushtail possum (*Trichosurus vulpecula*) is one of the few native mammal species found free-ranging at high population densities in zoos throughout Australia. This study sought to examine the possible role of possums in the transmission dynamics of *T. gondii* by assessing all potential hosts within a zoo setting. In the first instance we sought to compare the seroprevalence of antibodies to *T. gondii* amongst possums that occupied a metropolitan zoo with a woodland population maintained in a felid-free enclosure. Secondly, we assessed the frequency of predation of free-ranging possums by captive carnivores to establish if this pathway may be a viable route of *T. gondii* transmission. To verify if this interaction increased exposure to *T. gondii*, we assessed the seropositivity of captive carnivores that prey on possums. Lastly, we sought to investigate the seroprevalence of antibodies to *T. gondii* amongst captive felids to clarify their role as a possible source of the parasite within the zoo environment.

2. Materials and methods

2.1. Study sites

Possum serum samples were collected at two study sites. The first, Taronga Zoo, was located in an urban area in the northern Sydney suburb of Mosman, Australia (33°50′S 151°14′E). The zoo is inhabited by an abundant free-ranging possum population and houses over 300 exotic and native species including 6 felid species; the Asiatic golden cat (*Felis temmicki*), fishing cat (*Felis viverrinus*), clouded leopard (*Neofelis nebulosa*), lion (*Panthera leo*), sumatran tiger (*Panthera tigris sumatrae*) and snow leopard (*Panthera uncia*). The zoo perimeter had an electrified fence to deter stray cats and dogs from entering the grounds, however it did not restrict the movement of possums, which used over-hanging branches to move in and out of the zoo. Although this route may have also facilitated the movement of feral cats into the zoo, they have not been a documented problem on zoo grounds. The second site of possum serum collection was located in a non-urban area within Jenolan Caves Reserve Trust land, Blue Mountains, Australia (33°48′S 125°29′E). The reserve was characterised by eucalypt woodland and was inaccessible to the public. The site was bound by fencing to prevent invasion by exotic species and control of feral cat populations was performed throughout the enclosure. Ethics approval for this project was obtained from the Macquarie University Animal Ethics Committee (#2004/16), the Zoological Parks Board of New South Wales Animal Care & Ethics Committee (#4c/08/04) and the NSW Department of Environment, Conservation & Climate Change (#S11107).

2.2. Trapping

Possum traps were laid on a monthly basis between February 2005 and May 2006 in Taronga Zoo (16 trapping sessions) and at Jenolan Caves during October 2005 and May 2006 (2 trapping sessions). Possums were captured in cage traps (59 cm × 22 cm × 22 cm) baited with apples and a rolled oats/peanut butter mixture and transported either to the Veterinary and Quarantine Centre, Taronga Zoo or sampled on site at Jenolan Caves. All possums were permanently implanted with a passive integrated transponder (Microchips Australia, Victoria) to enable identification

of recaptured animals. Recaptured possums were included in the survey if the time since original capture exceeded 3 months (to ensure individuals were only sampled seasonally).

2.3. Sample collection

Animals were anaesthetised via a face-mask using 5% isoflurane in oxygen and basic biological data was collected including body measurements, weight, sex and age. Age was estimated from the degree of wear on the molar tooth (Winter, 1980). Skeletal body length was measured from the tip of the nose to the cloaca, whilst body mass (BM) was measured on electronic scales to the nearest gram. Body condition (BC) was calculated by dividing BM by the skeletal body size (BS) of possums (BC = BM/BS). This provided an estimate of body condition previously validated for the closely related mountain brushtail possum, *Trichosurus caninus* (Viggers et al., 1998). 5 mL of blood was drawn from the lateral tail vein and placed into serum clot activator tubes (Interpath Services, Sydney). After clotting, the samples were centrifuged at $200 \times g$ for 10 min, serum was collected in microtubes and upon return to the laboratory was frozen at $-80\ ^{\circ}C$ until analysis.

Serum was obtained from all 6 captive felid species held at the zoo (23 individuals) and all other captive carnivorous species known to prey on free-ranging possums including the leopard seal, *Hydrurga leptonyx* (2 individuals), chimpanzee, *Pan troglodytes* (16 individuals), saltwater crocodile, *Crocodylus porosus* (2 individuals) and dingo, *Canis lupis* (3 individuals). Anecdotal evidence from zoo staff, supported by physical evidence of possum carcasses in animal enclosures, indicated that these species were predators of possums. Blood was collected during routine health checks or medical procedures between 2002 and 2007 and prepared for screening as described above.

2.4. Serology

Serum was assayed for the presence of Ig-G antibodies to *T. gondii* using a modified agglutination test (MAT) as previously described (Dubey and Desmonts, 1987). Sera found to be positive for antibodies were diluted with phosphate buffered saline twofold starting at 1:25 to determine the highest dilution at which agglutination occurred. A MAT titre of 25 was regarded as indicative of exposure to *T. gondii*, based on validation studies by Dubey (1997). All serological assays were performed at the Animal Parasitic Disease Laboratory, Beltsville, MD, USA.

2.5. Post-mortem examination

Post-mortem examinations were performed on all possums found dead on zoo grounds and identified by the presence of a microchip as part of the study population. Gross pathology was performed in all cases, except when possums were too decomposed to carry out necropsy.

2.6. Statistical analysis

Seroprevalence was determined using data from possums at their first capture only and was calculated as the percentage of seropositive individuals in the population. Prior to statistical analysis, the possum population was pooled into three age categories; <2 years, 2–5 years and >5 years corresponding respectively to the reproductive maturity of possums: immature, sexually developed and post-reproductive. χ^2-Analysis was performed for seropositive possums to assess the influence of sex and age on the likelihood of *T. gondii* exposure. A one-way ANOVA was performed to determine the relationship between body condition of possums and exposure to *T. gondii*. For captive carnivores, a linear regression was carried out to determine whether age influenced the antibody titre. In addition, χ^2-analysis was performed to assess the influence of sex and age on the likelihood of *T. gondii* exposure for seropositive individuals. Analysis was performed using SPSS Version 15 software for Windows.

3. Results

A total of 126 individuals possums were captured at Taronga Zoo, many of which were recaptured during the trapping season, resulting in the collection of 168 serum samples (Table 1). Of the 126 individual possums in the population, 6 (4.8%) had detectable antibody titres to *T. gondii*. These 6 possums had titres of 1600 and were seropositive upon first capture. Three of the six seropositive possums were recaptured and had the same titre upon repeat testing. No seroconversion was observed during the 16-month study period. All 17 individual possums sampled at the woodland site tested seronegative for *T. gondii* antibodies.

There was no significant difference in exposure to *T. gondii* between possums of different sex ($\chi^2 = 0.111$, d.f. = 1, $n = 6$, $P = 0.739$) or age class ($\chi^2 = 4.667$, d.f. = 2, $n = 6$, $P = 0.097$). No possums below the age of 2 years were found to have produced antibodies against *T. gondii*. Possums that had been exposed to *T. gondii* exhibited significantly higher body condition than

Table 1
Monthly *T. gondii* seroprevalence data for possums captured at Taronga Zoo

Date	First capture		Recapture		Monthly seroprevalence
	No. of captured	Seropositive	No. of recaptured	Seropositive	
February 2005	12	1	0	0	8.3% (1/12)
March 2005	13	1	0	0	7.7% (1/13)
April 2005	8	1	0	0	12.5% (1/8)
May 2005	5	0	2	0	0% (0/7)
June 2005	8	0	2	1	10% (1/10)
July 2005	9	0	1	0	0% (0/10)
August 2005	12	1	2	1	14.3% (2/14)
September 2005	5	0	3	0	0% (0/8)
October 2005	10	1	2	0	8.3% (1/12)
November 2005	7	0	3	0	0% (0/10)
December 2005	7	0	4	1	9.1% (1/11)
January 2006	8	0	3	0	0% (0/11)
February 2006	8	0	4	0	0% (0/12)
March 2006	5	0	4	0	0% (0/9)
April 2006	7	1	6	0	7.7% (1/13)
May 2006	2	0	6	0	0% (0/8)
Total	126	6	42	3	4.9% (9/168)

possums that had not come into contact with the parasite ($F = 5.676$, d.f. = 1, $n = 126$, $P = 0.018$). A summary of the biological characteristics for each of the six seropositive possums is presented in Table 2.

Post-mortem examination of micro-chipped possum carcasses ($n = 13$) from the zoo grounds indicated that the leading cause of death was predation by a captive carnivore (31%, $n = 4$) followed by car collision (23%, $n = 3$) or other physical impact (23%, $n = 3$). Death due to bacterial infection (15%, $n = 2$) and chronic dermatitis necessitating euthanasia (8%, $n = 1$) were the least common causes of mortality of zoo possums. None of the six possums that were seropositive for *T. gondii* were amongst the animals found dead during the study.

Of the captive felids, 16 of 25 (64%) individuals were seropositive and all 6 species contained seropositive individuals. Seroprevalence ranged from 50 to 100% of individuals within each species. Antibody titre was positively related to the age of the host ($R^2 = 0.310$, $n = 25$, $P = 0.006$). However seroprevalence was not influenced by age ($\chi^2 = 5.38$, $n = 16$, $P = 0.717$) or sex ($\chi^2 = 0.25$, $n = 16$, $P = 0.617$).

Of the other captive carnivores examined, 5 of 23 individuals (22%) and 2 of the 4 species were seropositive for *T. gondii* antibodies. Seroprevalence averaged 13% amongst the chimpanzee population (2/16) and 100% amongst the dingo population (3/3). Antibody titre was not related to age of the host ($R^2 = 0.100$, $n = 23$, $P = 0.162$). Nor was seropreva-

Table 2
Demographic characteristics of the six *T. gondii* seropositive possums captured at Taronga Zoo

Seropositive individual #	Sex	Capture event	Date of capture event	Age (years)	Body condition (%)	Average body condition of seronegative possums[a] (%)
1	Male	1	February 2005	2–5	83.33	73.83
2	Male	1	March 2005	2–5	72.11	73.83
		2	June 2005	2–5	78.38	73.83
		3	August 2005	2–5	85.71	73.83
3	Male	1	April 2005	2–5	85.29	73.83
4	Female	1	August 2005	2–5	82.57	65.19
		2	December 2005	2–5	72.56	65.19
5	Male	1	April 2006	>5	62.65	80.97
6	Female	1	October 2006	<5	61.76	56.41

[a] Calculated by averaging body condition of seronegative possums ($n = 159$) according to different age–sex groups.

lence effected by age ($\chi^2 = 0.60$, $n = 5$, $P = 0.896$) or sex ($\chi^2 = 1.80$, $n = 5$, $P = 0.180$). The serological status and demographic characteristics for all captive animals is presented in Table 3.

4. Discussion

Compared to other serological assays, the MAT has proven to be the most sensitive method of *T. gondii*

Table 3
Seroprevalence of *T. gondii* in captive carnivores from Taronga Zoo collection

Species	Individual	Sex	Age (years)	Titre	Seroprevalence
Asiatic golden cat (*Felis temnicki*)	Valentin	M	2	<25	50% (1/2)
	Nugi	M	12	>200	
Fishing cat (*Felis viverrinus*)	Beranang	M	1	<25	50% (2/4)
	Cantik	F	2	<25	
	Fiddle	F	5	>200	
	Pakikan	M	8	>200	
Clouded leopard (*Neofelis nebulos*)	Nonah	F	12	>200	100% (2/2)
	Samar	M	13	>200	
Lion (*Panthera leo*)	Kuchani	F	3	25	100% (3/3)
	Njeri	F	3	50	
	Shinyangi	F	3	25	
Sumatran tiger (*Panthera tigris sumatrae*)	Jumilah	F	1	<25	67% (4/6)
	Dumai	M	1	>200	
	Sendiri	M	1	<25	
	Asiqua	F	3	25	
	Selatan	F	12	100	
	Shiva	M	17	>200	
Snow leopard (*Panthera uncia*)	Kamala	F	1	<25	67% (4/6)
	Leon	M	2	>200	
	Sabu	F	2	>200	
	Samara	M	2	<25	
	Prafula	M	14	>200	
	Omaha	F	14	50	
Leopard seal (*Hydrurga leptonyx*)	Rove	M	1	<25	0% (0/2)
	Diesel	M	13	<25	
Saltwater crocodile (*Crocodylus porosus*)	–	F	Unknown	<25	0% (0/2)
	–	M	Unknown	<25	
Chimpanzee (*Pan troglodytes*)	Furahi	M	2	<25	13% (2/16)
	Lana	F	3	<25	
	Shikamoo	M	4	<25	
	Samaki	M	4	<25	
	Sheba	F	7	200	
	Sandali	M	10	<25	
	Chimbuka	M	11	<25	
	Kamili	F	12	<25	
	Shabani	M	12	<25	
	Lubutu	M	13	<25	
	Kuma	F	14	<25	
	Shona	F	19	<25	
	Lisa	F	26	<25	
	Sasha	F	27	<25	
	Koko	F	31	<25	
	Spitter	F	46	>200	
Dingo (*Canis lupis*)	Sugar	F	13	25	100% (3/3)
	Sally	F	14	50	
	Jack	M	14	>200	

antibody detection for marsupial sera (Johnson et al., 1989; Hartley and English, 2005). Using this technique, this study demonstrated that free-ranging possums inhabiting Taronga Zoo had come into contact with and produced Ig-G antibodies to *T. gondii*, however possums from woodland habitat remote from urban areas had not. These findings parallel a comparative study of feral pigs from a mainland and cat-free island habitat (Dubey et al., 1997), highlighting that wildlife living with sympatric populations of felids are at high risk of exposure to *T. gondii*. While the exact source of *T. gondii* could not be identified in this study, two felid populations may have contributed to environmental loading of oocysts in the urban area and subsequent exposure of possums; the captive felid population housed at Taronga Zoo or domestic and stray cats from outside the zoo environs.

Investigation of the captive felids at the zoo indicated that two-thirds of the population had come into contact with *T. gondii* and that all felid species were seropositive, implicating them as a potential reservoir within the zoo setting. Consistent with these findings, studies from zoos worldwide demonstrate a high seropositivity amongst captive felids (76%: Lappin, 1991; 83%: Zhang et al., 2000; 93%: Sedlak and Bartova, 2006; 55%: Ramos Silva et al., 2007). The plentiful opportunities to prey on pest rodent populations and the practice of feeding carnivores raw rabbit or livestock from breeding centres may promote infection of captive felids within zoos (Lukesova and Literak, 1998). In Australian zoos, the diet of captive felids may also include raw kangaroo meat, a recognised source of infection for humans and animals (Johnson, 1996). In this study, the increase in antibody titre with age of the felid indicated that opportunities for exposure to the parasite were cumulative over the animal's life span, suggesting that zoo conditions may be favourable for the persistence of *T. gondii*.

Exposure of possums to *T. gondii* may also have resulted from interaction with domestic or stray cats from the surrounding neighbourhood. Monitoring of nocturnal movements of zoo possums revealed that many individuals possessed home ranges encompassing the adjacent residential areas (Hill, unpublished data) where at least 24% of residents owned a cat that spent time outdoors after dark (Hill et al., 2007). A previous study of *T. gondii* carried out in residential Sydney reported a similar seroprevalence (6.3%) in urban possums (Eymann et al., 2006) as that observed in this study (4.8%). Comparison of these results highlights that possums were at a similar risk of exposure regardless of whether they inhabited residential neighbourhoods or zoo grounds.

The seropositivity of possums that inhabit zoo grounds raises questions about the potential for transmission of *T. gondii* to the captive collection via predation of infected individuals harbouring tissue cysts. While predation by captive carnivore was a prevailing cause of death for possums inhabiting the zoo, serological screening revealed that of the four other carnivorous species tested, only the chimpanzee and dingo had come into contact with *T. gondii*. The seroprevalence of dingoes, a natural predator of possums in the wild, was much higher than chimpanzees, which may reflect their superior hunting efficiency. While carnivory may be responsible for the exposure of dingoes to *T. gondii* and felids such as the lion, golden cat and tiger that also regularly prey on possums, the low seroprevalence of possums implies that, despite their abundance at the zoo, only a small number potentially act as intermediate hosts. Consequently, high numbers of possums must be caught and consumed before carnivores would be at risk of ingesting the tissue cysts of an infected animal.

The low prevalence observed in possums corresponds with many other serological studies of *T. gondii* in free-ranging marsupial populations. For example, exposure to the parasite was found in 8% (2/25) of tammar wallabies, *Macropus eugenii* (Jakob-Hoff and Dunsmore, 1983), 4% (6/152) of Bennett's wallabies, *Macropus rufogriseus rufogriseus*, 1.2% (1/85) of Tasmanian pademelons, *Thylogale billardierii* (Johnson et al., 1989) and 6.7% (10/150) of Eastern barred bandicoots, *Perameles gunnii* (Obendorf et al., 1996). The low seropositivity of these populations would suggest that unlike rodents (Berdoy et al., 1995), marsupials are not exploited by *T. gondii* as an intermediate host vital in the perpetuation of the parasite's life-cycle. Due to the separate evolution of cats and marsupials, *T. gondii* has had limited opportunity to expand its intermediate host range to include Australian fauna (Innes, 1997). Typical of hosts that have not developed immunological defences to the parasite, marsupials are highly susceptible to fatal toxoplasmosis (Innes, 1997). Interestingly, seropositive possums in this study showed enhanced body condition, suggesting that only individuals with superior fitness had the capacity to mount resistance to the parasite and survive, while those that were less fit may have succumbed to fatal infection.

In conclusion, possums were not identified as an important intermediate host of *T. gondii* in the zoo setting and therefore were believed to present minimal risk to the health of zoo animals. Preventative measures may however be useful to ensure conditions do not become conducive for the transmission of *T. gondii* between free-ranging and collection populations. Practices such as removing faeces from felid enclosures

before nightfall when nocturnal wildlife emerge to forage, may restrict opportunities for ingestion of oocysts. As the birth of kittens usually corresponds with a peak in *T. gondii* oocysts shedding (Dubey and Carpenter, 1993) this measure may be most effective for breeding populations. In addition, the cutting of overhanging branches may exclude arboreal wildlife from moving between residential areas and the zoo during nocturnal ranging. These measures, by aiming to disrupt the *T. gondii* life-cycle may also help to maintain the health of captive animal and free-ranging possum populations, both of which utilise zoos as an important habitat in the urban setting.

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